Original Article

Serum melanin and alpha-melanocyte stimulating hormone levels in Pakistani individuals affected with oculocutaneous albinism

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Objective: To determine the serum levels of melanin, α-MSH, and to find their association and correlation in patients with Oculocutaneous albinism (OCA).

Methodology: This study included 80 subjects; 40 OCA patients and 40 healthy controls. ELISA was performed to determine the serum concentrations of melanin and α-MSH.

Results: Serum melanin level of6.0 ng/dL was significantly reduced and serum levels of α-MSH of 9.8 ng/dL was significantly higher ($p < 0.05$) in patients as compared to healthy controls who had 14 and 3.48, respectively.

Conclusion: Serum melanin levels were found low with a compensatory rise in α-MSH levels in OCA patients as compared to controls.

Keywords: Oculocutaneous albinism (OCA), Melanin, alpha-melanocyte stimulating hormone, α-MSH.

INTRODUCTION

Oculocutaneous albinism (OCA) is a rare genetic disorder characterized by an absolute deficiency or abnormal production of melanin. Melanin comprises of two types; eumelanin (blackish-brown pigment) and pheomelanin (yellow pigment). It gives a wide range of colors to hair, skin and eyes. Melanin deficiency in OCA causes light-colored hair (white, brown, golden instead of black color), skin (white, reddish-white), and various ophthalmological features like photophobia, nystagmus, reduced visual acuity and foveal hypoplasia due to deficiency of pigments in the retinal pigment layer. OCA develops due to the mutation in specific genes, which are involved in melanin synthesis inside the melanocytes.[1] Mutations in six genes (*TYR, P, TYRP1, SLC45A2, SLC24A5, C10 or f11*) and one locus (*4q24*) are involved in non-syndromic OCA (nsOCA1-7).[2]

Melanin synthesis occurs in melanocytes of skin and other tissues and is regulated by several factors such as bioactive peptides like alpha-melanocyte-stimulating hormone (α-MSH) and its intermediary form, adrenocorticotropic hormone (ACTH).[3] The α-MSH gets attached with the MC1R receptor and stimulates transcription factor MITF that in turn activates TYRP1 enzyme and other proteins involved in melanogenesis.[4,5] Tyrosinase, a copper-containing enzyme, and two closely related zinc-containing enzymes tyrosinase-related proteins (TYRP1 and TYRP2) are involved in melanogenesis.[6] Tyrosinase enzyme catalyzes the first two reactions of the melanogenesis pathway: hydroxylation of tyrosine forms L-3, 4-dihydroxyphenylalanine (L-DOPA) and subsequent oxidation of L-DOPA produces dopa-quinone (Fig. 1). Genetic or biochemical defects in tyrosinase and related enzymes of the melanogenesis pathway cause decreased or absolute deficiency of pigmentation of the skin, hair, and eyes.[2]

Dermal melanin not only imparts color to the skin but also protects the skin from the harmful effects of ultraviolet radiation. Melanin granules in the retinal pigment epithelium (RPE) protect the RPE cells from harmful effects of oxidative stress and could be involved in the degradation of rod outer segments (ROS) of RPE as part of lysosomal degradation pathways.[7,8] Despite of much significance in pigmentation of various body parts, the levels of melanin and α-MSH in OCA patients have not been determined. The present study was aimed to determine the levels of melanin and α-MSH in OCA in the Pakistani population and correlate their levels with different phenotypic features of OCA.

METHODOLOGY

In this cross-sectional comparative study, OCA families were recruited from Services Hospital, Ittefaq Hospital and Layton Rehmatullah Benevolent Trust (LRBT) Eye Hospital, Lahore Pakistan. The study was approved by Advance studies and Research Board of University of Health Sciences, Lahore and written informed consent was taken from all participants.We included 20 families with total 40 affected cases, and the 46 age-matched healthy individuals as controls.

Clinically diagnosed cases with hallmark features of OCA like decreased visual acuity, photophobia, nystagmus, foveal hypoplasia with light color skin and hair) were included and exclusion criteria was syndromic albinism and ocular albinism. The complete demographic data like name, age, gender, the colour of skin, hair, and iris, skin rashes, and visual acuity were taken from their hospital records.

The field visits were done for the history taking and 5.0 ml of venous blood was drawn. To measure levels of melanin and α-MSH, commercially available ELISA kits Cusabio Biotech Co., Ltd, Wuhan Fine Biological Technology Co. Ltd, China) were used.

Statistical Analysis: The data were analyzed using SPSS version 22. Shapiro-Wilk test was applied to document the distribution of the data. For normally distributed data, Independent sample t-test and Pearson correlation analysis were applied while Mann-Whitney U-test and Spearman correlation analysis were performed to compare variables between patients and controls and to find correlation respectively. One way analysis of variance was applied to compare study variables in groups based on skin color, hair color, and skin rashes. $p < 0.05$ was considered significant.

RESULTS

The median age of patients and controls was the same as represented in Table 1. The mean serum level (Mean ± S.D) of melanin was significantly lower ($p = 0.001$) in patients (6.0 (1.0 – 13.5) ng/dL) than controls 14 (9.0 – 24.75) ng/mL), while the mean serum α-MSH levels were significantly higher ($p = 0.001$) in patients 9.8 (6.62 – 16.79) ng/mL than in the controls 3.48 (2.80 – 5.80) ng/mL. In male OCA patients, the white hair were more likely (35%) to be as compared to female patients (22%), but was statistically insignificant ($p > 0.05$). The reddish-white skin colour (45%) and skin rashes (50%) were more likely to be present in male OCA patients as compared to females OCA patients (20%) and (30%) respectively, which were statistically significant (p-value = 0.05 and 0.045) (Table 2).

Melanin levels increases with age which is noted in different types of OCA (Fig. 1). The one-way analysis of variance (ANOVA) was used to determine whether there were any statistically significant differences present among the means of independent (unrelated) groups like hair color (Golden, blonde, white), skin color (red, reddish-white, white), skin rashes (No rashes, mild on sun exposure, severe on sun exposure) and iris color with dependent variables like serum Melanin and α-MSH levels. Serum melanin level was statistically significant between hair color of OCA patients with F-value = 5.174 and p-value 0.004 (white hair have less melanin as compared to colored hair), while α-MSH has no association with the hair color of albinism patients ($p > 0.05$). In OCA patients, serum melanin levels were high (16.55.0 ± 9.1) in reddish colour skin albinos, as compared to white skin albinos (6.75 ± 5.9) but the relationship was not statistically significant, while the skin rashes have no significant associations with levels of melanin and serum α-MSH levels (Table 3). In the patients group, a positive Spearman rho correlation was observed between serum melanin and age ($r = 0.478$, p-value = 0.002).

Table 1: Demographic and laboratory data of patients and controls.

Parameters		Patients (n = 40)		Controls (n = 40)		p-value
		Mean ± S.D	Median (Q1 – Q3)	Mean ± S.D	Median (Q1 – Q3)	
Age		17.8 ± 14.98	14.0 (9.0 – 24.75)	18.08 ± 14.54	14 (9.0 – 24.0)	0.80†
Melanin		7.4 ± 7.21	6.0 (1.0 – 13.5)	17.81 ± 14.98	14(9.0 – 24.75)	0.001*
α-MSH		11.61 ± 6.8	9.8 (6.62 – 16.79)	5.0 ± 4.14	3.48 (2.80 – 5.80)	0.001*
Corrected Visual Acuity	**R**	0.77 ± 0.35	0.70 (0.60 – 0.97)	-------	---------	------
	L	0.77 ± 0.35	0.70 (0.60 – 0.97)	-------	---------	------

p-value ≤ 0.05 is considered significant, † p-value > 0.05 is considered non-significant, IQR; interquartile range

Table 2: Association of clinical phenotype in patients with OCA.

Characteristic	Categories	Male (25)	Female (15)	Statistics	
				x^2	p-value
Hair Color	White	14 (35%)	9 (22.5 %)	3.5	0.32
	Golden	8 (20%)	5 (12.5 %)		
	Blonde	3 (7.5%)	1 (2.5 %)		
Skin Colour	Reddish White	18 (45%)	8 (20 %)	3.9	0.05*
	White	5 (12.5%)	7 (17.5 %)		
	Red	2 (5%)	0		
Skin Rashes	No rashes	5 (12.5%)	3 (7.5 %)	6.1	0.045*
	Mild rashes on sunlight exposure	16 (40%)	7 (17.5 %)		
	Severe on sunlight exposure	4 (10%)	5 (12.5 %)		
Iris Colour	Grey	22 (55%)	13 (32.5%)	0.015	0.99
	Brown	3 (7.5%)	2 (5 %)		

Table 3: Melanin levels and α-MSH levels.

		Sum of Squares	df	Mean Square	F	p-value
Serum Melanin Levels	Between Groups	612.049	3	204.016	5.174	0.004*
	Within Groups	1419.429	37	39.429		
	Total	2031.478	40			
Serum α-MSH Levels	Between Groups	223.168	3	74.389	1.692	0.186
	Within Groups	1583.166	37	43.977		
	Total	1806.334	40			

*P-value ≤ 0.05 is considered significant, Df; Degree of freedom, F; test statistic of Anova

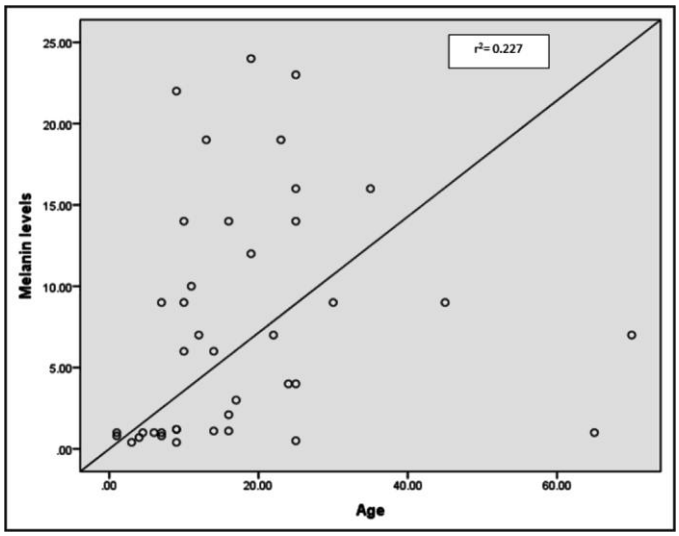

Fig. 1: Correlation of melanin with patients' age.

DISCUSSION

Melanin plays a vital role in the synthesis of retinal pigment epithelium with co-activity of various trace elements. In the present study, significantly low serum melanin and high serum α-MSH levels were found in patients compared to the controls (p = 0.001). An increase in the α-MSH levels was found in the patients with decreased levels of melanin indicating a strong reciprocal association between serum melanin and α-MSH.

Type 1A OCA patients have an absolute deficiency of melanin while in other OCA types (OCA1B – OCA7) variable levels of melanin were found. It has been postulated that some melanin pigment continues to develop in these patients which resulted in increased melanin levels with advancing age.[3,9,10] Also, decreased levels of melanin are directly related to the decrease in

the vision.[11,12]

Yakimov et al has studied the Melanin distribution and transport in the dermal-epidermal layers by fluorescence and micro spectroscopy techniques.[13] Schidlowski et al reported that genetic defects in melanin biosynthesis are directly linked to increased sensitivity to the skin cancer, most commonly squamous cell carcinoma.[14]

Decreased melanin concentration leads to visual defects like iris trans-illumination, refractive errors, decreased visual acuity (VA) and nystagmus which badly affect the quality of life.[11,13] This study presents the addition of data and is the first-ever report from Pakistan about the studies of melanin and α-MSH in albinism patients.[15,16] Evaluation of this type of data may enhance the understanding of biological pathways to the clinicians for the disease diagnosis and management. Further study should be conducted on large sample size with some additional biochemical variables of the disease pathway for the better understanding of disease mechanism.

CONCLUSION

Levels of serum melanin were significantly reduced in OCA patients as compared to healthy controls while the levels of serum α-MSH are increased in the patients as compared to healthy controls. The melanin levels are positively correlated with the advancing age, and no correlation was observed for α-MSHin OCA patients.

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Conflict of Interest: None declared.
Rec. Date: Feb12, 2021 Revision Rec. Date: Jun 4, 2021 Accept Date: October 14, 2021.

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